Jellyfish Venom and Toxins: A Review

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Abstract

Although countless numbers of people are being stung every year by poisonous jellyfish throughout the world, statistical data is rarely available, even for fatal cases, except for Australia. There has been a significant amount of research for the treatment of cnidarian stings, but confusion still exists as to what is the most effective first aid and clinical management. In addition, only a few toxic components of jellyfish venoms have been identified so far, suggesting it is one of the most neglected areas in toxinology research. This is probably because jellyfish are difficult to obtain; many species are randomly distributed in the wide open sea, compared with other venomous animals. Secondly, unlike the milking of snake venom, it is extremely difficult to collect jellyfish venom of high purity without contamination of other tissue debris. Thirdly, all the toxins identified from jellyfish venoms until now are proteins, and many are intrinsically susceptible to and easily denatured by harsh environmental conditions (e.g., temperature, pH), resulting in the loss of their biological activities during the extraction and purification procedures. Therefore, the isolation and characterization of jellyfish toxins has lagged behind that of many other animal toxins. A few protein toxins have been reported, mainly from box jellyfish (Cubozoa) species. In this chapter, various jellyfish venoms that have been investigated are reviewed, as well as the protein toxins which have been identified to date.

Introduction

Over the last decade, a dramatic increase in global jellyfish blooms has been observed due to climate change and other anthropogenic disturbances. Jellyfish blooms can induce direct negative effects on human enterprise, including reduction of fish populations, disturbance of fishing by tearing of nets, human stingings, and clogging of coastal power plant cooling water intakes, leading to enormous economic loss.

Jellyfish belong to the phylum Cnidaria, which can be divided into five classes, Anthozoa, Cubozoa, Hydrozoa, Scyphozoa, and Staurozoa. Except for anthozoans, most cnidarians have bells of different shapes and sizes and take the form of a free-floating medusa, the form which is commonly referred to as a jellyfish. Most jellyfish are relatively harmless to humans and some of them, typically in Asian countries, also are used for food. Jellyfish have special cells, nematocytes, which contain stinging organelles called nematocysts, that are used for prey capture, defense, and locomotion. Nematocysts have a spiralized thread immersed in the jellyfish venom. When nematocytes sense a chemical or a mechanical stimulus, the coiled thread everts, injecting venom into the prey or attacker (Fig. 1). Tentacles contain from a few 1000 to several billion nematocysts that discharge into the skin within a fraction of a second and can paralyze and kill predators (mainly zooplankton and small fish) (Malej 1982).

Furthermore, jellyfish can significantly impact human activities and ecosystems due to their envenomation. Although most jellyfish can cause envenomation, some

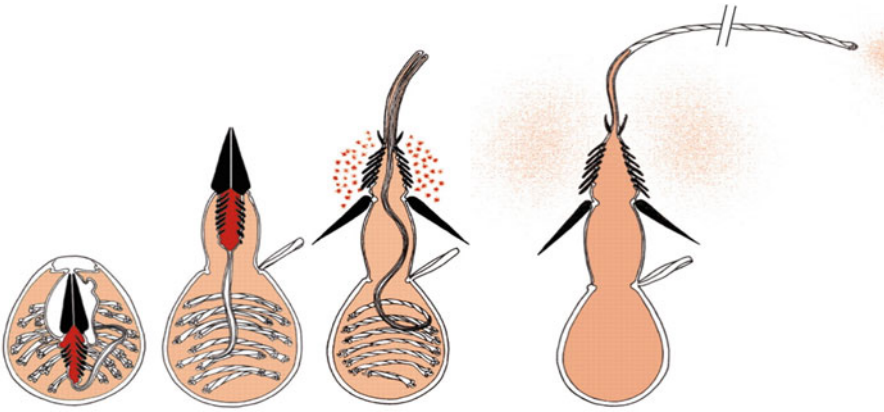

Fig. 1 Nematocyst discharge scheme. Nematocysts contain coiled threads that may bear barbs. The outer wall of the nematocyte (now shown) has hair-like projections called cnidocils that are sensitive to touch. When touched, the nematocysts are known to fire coiled threads that can either eject rapidly into the flesh of prey or predators or ensnare it. These coiled threads release toxins into the target (From Szczepanek et al. 2002)

species fail to inject sufficient amount of venom deep into skin layers or produce toxins that do not cause significant harm to humans (Barnes 1960). Human envenomation by jellyfish can induce a large variety of symptoms, ranging from a slight local effect to life-threatening symptoms (Cegolon et al. 2013). In humans, jellyfish can cause severe envenomations with extensive dermonecrosis, edema, diffused neurotoxicity, motor and respiratory problems, cardiovascular symptoms, hypotension, and even death. In particular, the cubozoan *Chironex fleckeri* (sea wasp) can cause serious cardiotoxicity within a few minutes, and several deaths have been reported along the Australian coastline during the last century (Brinkman and Burnell 2009). Additionally, some people are susceptible to allergic reactions, including immediate and delayed-type hypersensitivity responses (Tibballs et al. 2011). Stings of prominent species (*Physalia physalis*, *Chironex fleckeri*, *Carybdea arborifera*, *Carukia barnesi*, and *Pelagia noctiluca*) inject into the skin tubules which are composed of mini-collagens, glycoproteins, and polysaccharides, all of which can work as immunogens. It is well known that jellyfish stings are a particularly serious health problem along some Asian and Australian coasts and in Pacific archipelagos (Feng et al. 2010). Recently, the occurrence of venomous jellyfish species is becoming more common in the East Asia area (Kang et al. 2014).

Jellyfish venom is a mixture of toxins with a wide range of biological activities producing hemolytic, insecticidal, cardiovascular, antioxidant, and cytotoxic effects (Kim et al. 2006; Li et al. 2014; Kang et al. 2009). Various studies have revealed that cnidarian venoms contain vasoactive compounds such as 5-hydroxytryptamine (5-HT), catecholamines, histamine, neuroactive compounds such as quaternary ammonium compounds and certain amino acids and small peptides, and proteins, including proteases, phospholipases, and cytolytic and

hemolytic agents. The components of jellyfish venoms and their mechanism of actions are still far from being understood.

This review will focus on the toxins or partially purified bioactive fractions that have been isolated from jellyfish venom to date. First, their effects on human health will be considered, based partly on in vitro characterization of the biological activities of their venoms. Second, identification and purification of jellyfish venom components will be presented. Third, current protocols for the management of jellyfish stings, depending on the jellyfish species, will be described. Last, biological activities of jellyfish venom with potential medical and therapeutic activity will be reported.

Envenomation

Cubozoa

1. Cubozoans are the most venomous jellyfish and are named "box jellyfish" because of their cube-shaped bell. This class is composed of two orders, Carybdeida and Chirodropida. Carybdeida have four small tentacles, while Chirodropida are multitentacled. Carybdeida of medical importance are *Carukia barnesi*, *Tamoya* spp., and *Carybdea rastoni*. Contact with jellyfish of the order Carybdeida typically induces local cutaneous inflammation with invariably linear lesions (small blisters, erythema, and wheals) and usually, but not always, immediate pain (Cegolon et al. 2013). Erythema and wheals may appear within minutes and last several days, while moderate pain may last up to 2 h, but slight pigmentation at the sting site is observed for at least 2 weeks (Cleland and Southcott 1965). Contact with other jellyfish in this order can also induce the Irukandji syndrome. This syndrome is described as a set of delayed systemic effects. Severe symptoms appear on average 30 min after contact with the jellyfish and can last for several days (Carrette et al. 2012). Among suspected Irukandji species, *Carukia barnesi* and *Alatina mordens* are known to produce Irukandji syndrome, but it is not confined to stings by these species (Winter et al. 2008). While local effects of the sting are rarely noticed and may appear as a perceptible erythema, the systemic symptoms that include vomiting, nausea, intense muscle pain, cramps, lower back pain, headache, sweating, agitation, restlessness, and sometimes hypertension start about 30 min after contact with jellyfish. Two of 62 victims presented severe cases with systemic symptoms and tachycardia and hypertension. After 1.5–18 h, pulmonary edema and reduced cardiac output were observed (Carrette et al. 2012). The deaths in 2002 of two tourists in North Queensland, Australia, highlighted the potential risk and severity of this syndrome. Fenner et al. stated that this syndrome may be caused by an excess of circulating catecholamines. However, this mechanism has not been confirmed in human envenomation (Fenner and Hadok 2002). *Tamoya* spp. jellyfish are called "fire jellyfish." They are relatively large carybdeid cubozoans and characteristically have a taller, transparent bell. Unlike other smaller

carybdeids, the symptoms that develop from this jellyfish sting are similar to Irukandji syndromes, and the lesions that may occur include skin necrosis, vesicle, and pruritus.

In the order Chirodropida, *Chironex fleckeri*, the Australian box jellyfish, is known to be the most dangerous jellyfish in the world (Tibballs 2006). At least 63 victims had sudden deaths in Australian waters up to 1994. It has been found in northern Australia and Southeast Asia. Contact with its tentacles induces a local cutaneous inflammatory reaction which is very painful and can persist for several days. The victim may scream and become irrational. The stung skin areas were large and edematous and contained erythematous wheals which became blisters and which progressed to skin necrosis during the healing process. When the skin makes contact with large *C. fleckeri with a diameter of 15 cm or more*, severe symptoms can also be observed, including an excruciating pain, dyspnea, unconsciousness, hypertension, hypotension, and cardiopulmonary arrest. *Chironex fleckeri* venom was demonstrated to induce a direct toxic effect on cardiac and vascular tissues, effects not yet confirmed for the purified lethal components (Hughes et al. 2012). Death can occur within minutes after the envenomation, as a result of cardiac and respiratory effects (Tibballs 2006). Sometimes, envenomation of *C. fleckeri* can cause immunological responses due to delayed hypersensitivity reactions; actually, 11 of 19 victims by *C. fleckeri* sting presented immunological effect which resolved spontaneously or with treatment of oral antihistamines and topical corticosteroids (O'Reilly et al. 2001).

Chiropsalmus quadrigatus, a smaller jellyfish than *C. fleckeri*, is a very common species in the Indo-Pacific ocean from Australia to the Philippines and Japan. Stings by this jellyfish produce intense pain immediately and erythema and swelling within a few minutes, and sometimes, these symptoms last for at least 24 h. Severe envenomations may cause hypertension, bradycardia, cardiac asystole, respiratory failure with pulmonary edema, shock, and even death. Death cases have been reported in the Philippines and Japan but not in Australia (Tibballs 2006; Nagai et al. 2002). However, its effects are less than envenomation by *C. fleckeri*.

Chiropsalmus quadrumanus is found in warmer water of the Atlantic Ocean from North Carolina (USA) to Brazil. Envenomation by this species can be harmful to the cardiovascular and respiratory systems and fatal (Nagai et al. 2002; Cegolon et al. 2013).

Hydrozoa

Physalia spp. are not true jellyfish but are considered as siphonophore jellyfish and belong to the class Hydrozoa. There are two species, *Physalia physalis* (the Portuguese Man O' War) and *Physalia utriculus* (the Blue Bottle). *Physalia physalis* is distributed worldwide and is larger than *P. utriculus*. *Physalia utriculus* is found in the Indo-Pacific, Indian, and South Atlantic oceans. *Physalia* spp. are dangerous jellyfish because they often are stranded on the beaches but are still able to sting and envenomate, even after several days of dehydration (Tibballs 2006).

Contact with *P. physalis* usually induces sharp pain, rapid development of vesicles, systemic effects with abdominal ache, nausea, vomiting, and spasms. By contrast, *P. utriculus* sting causes mild symptoms, such as local pain and very rarely systemic effects. It was observed in most cases that the marks and skin lesions at sting sites faded within 24 h. However, severe cases of *P. physalis* sting cause neurological (confusion, lethargy, and fainting) and cardiorespiratory (dyspnea, precordialgia (pain in the precordial region), and collapse) signs. *Physalia physalis* stings are the most common type of jellyfish stings in Australia, estimated to occur 10,000 times annually on the eastern coast (Fenner and Williamson 1996), but systemic symptoms are rarely observed (Tibballs 2006). However, deaths due to respiratory arrest and cardiovascular collapse have been reported.

Scyphozoa

Although Scyphozoa are known to be less dangerous to humans than jellyfish of the class Cubozoa, contact with *Cyanea capillata*, *Nemopilema nomurai*, *and Stomolophus meleagris* can lead to systemic symptoms (Cegolon et al. 2013). *C. capillata* (named "Lion's Mane") is very commonly found in the cold seas of the Northern Hemisphere including the North Sea and in Australia. The diameter of the umbrella ranges from 30 cm to 2 m. Contact with this jellyfish produces minor or more severe pain, discomfort, swelling, and erythema. Erythematous stripes may persist for several days. Systemic symptoms are unusual, but sometimes muscle cramps, sweating, dizziness, and nausea occur (Cegolon et al. 2013). No death cases have been reported until now.

Pelagia noctiluca is distributed worldwide, being found in both tropical and cold waters (Cegolon et al. 2013), and is very common in the Mediterranean Sea (Mariottini et al. 2008). Envenomation is not fatal but produces discomfort. Local effects include painful cutaneous reactions, including blistering, edema, and erythema. Lesions can heal within 2 weeks, but scarring and hyperpigmentation can remain. This lesion can be resolved with topical hydroquinone (Kokelj and Burnett 1990). Delayed or relapsing eruptions are sometimes observed at the site of contact. Systemic effects are very rarely observed. No deaths have been reported, but allergic reactions have been observed, with possible cross-reactivity with other jellyfish (Cegolon et al. 2013).

Aurelia aurita is found in temperate and temperate-cold waters around the world. It is known to be relatively harmless to humans or even non-stinging, but cases of local cutaneous reaction associated with pain have been reported (Tibballs 2006). There are significant stinging cases reported for *A. aurita* in the Gulf of Mexico (Burnett et al. 1988). In China, 18 of 136 victims stung by *A. aurita* developed severe symptoms (Cao and Shao 2001).

Rhopilema esculentum is as an edible jellyfish like its relative, *Rhopilema hispidum*, and is distributed widely in the East China Sea and Yellow Sea. In general, the first species produces significant pruritus and the second causes a persistent eruption. When a subject was stung by *R. esculentum*, instant mild pain

was experienced followed by a strong pruritus lasting 30 min before complete resolution (Kawahara et al. 2006).

Nemopilema nomurai is a large jellyfish with a bell size up to 2 m that is found in the East China Sea, Yellow Sea, and East Sea of Korea. Blooms have been more frequent in recent years. Common symptoms are cutaneous lesions with burning pain and erythematous eruptions with small vesicles, but not to be ignored are severe clinical symptoms (Kang et al. 2014). It also can cause cardiovascular depression in experimental animals (Kim et al. 2006) and may cause death in humans. In Chinese sea, 13 fatal cases by this jellyfish were reported (Zhang et al. 2005).

Molecular Constituents

Several jellyfish toxins have been characterized biochemically, toxicologically, and pharmacologically due to efforts of several laboratories during the last few decades. Box jellyfish (Cnidaria: Cubozoa: Cubomedusae) have been most thoroughly studied. For these reasons, several box jellyfish toxins are briefly described below.

Carybdea rastoni Toxins (CrTXs)

In 2000, two labile but potent hemolytic toxins, named as CrTX-A and CrTX-B, were purified from *Carybdea rastoni*, and their full-length cDNA sequences, which consisted of 18 amino acids as signal peptide and 432 in the complete open reading frames, were reported (Nagai et al. 2000b). These sequences were obtained for the purified toxins (which showed hemolytic activity) using peptide mapping with lysylendopeptidase and 5′/3′ RACE reaction from total RNA extracted from an intact tentacle. The two proteins were shown to be 43 and 46 kDa size by SDS-PAGE. After hydrolysis by lysylendopeptidase then separated into three fragments for CrTX-B and five fragments for CrTX-A. These fragments were identified using a PSQ-1 protein sequencer (Shimadzu, Japan) and compared with the deduced amino acid sequences. They suggested that the CrTX-A (43 kDa) toxin is modified CrTX-B (46 kDa), because CrTX-A exists only in nematocysts and CrTX-B exists only in tentacle extracts that were analyzed by the Western blot method. This supported the idea that CrTX-B is a modified form CrTX-A that is inserted into the nematocysts.

The toxicity of CrTXs varied based on the method of injection, but in case of intravenous injection into mice, the minimum lethal dose was less than 20–40 μg/kg within 40 min after injection, and it was less than 100 μg/kg within 8 h after an intraperitoneal injection. The minimum lethal dose of CrTX-A in crayfish after intra-hemocoel injection was less than 5 μg/kg. In addition, CrTX-A and CrTX-B hemolyzed 50 % of 0.8 % sheep red blood cells at concentrations of 1.9 and 2.2 ng/ml, respectively. The other toxic effects of CrTX found for partially purified *C. rastoni* toxin (pCrTX) were hemolysis, dermatonecrosis, plate aggregation, and

contractions in rabbit aorta (Azuma et al. 1986). Furthermore, partially purified *C. rastoni* toxin (pCrTX) induced contractions in rat aorta by release of endothelium-derived relaxing factor.

Carybdea alata Toxins (CaTXs)

Two hemolytic *Carybdea alata* toxins, toxin-A (CaTX-A, 43 kDa) and toxin-B (CaTX-B, 45 kDa), were purified by another method than for the purification of CrTXs, and their full-length cDNA sequences were determined (Nagai et al. 2000a). The deduced amino acid sequences have a putative signal peptide at the 18th amino acid position and a mature polypeptide of 445 amino acid residues. In a localization test of CaTX-A and CaTX-B, it was found out that only CaTX-A existed in the nematocysts and not CaTX-B. This indicated that the biochemical mechanisms of CaTX-A and CaTX-B processing were very similar with that of CrTXs of *Carybdea rastoni*. Alignment of the deduced amino acid sequence of CaTX-A had 43.7 % homology with CrTXs. By peptide mapping of CaTX-A, only the C-terminal region of CaTX-A had homology with CrTXs and not the N-terminal region. CaTX-A toxin showed an LD_{50} range of 5–25 μg/kg and hemolysis of sheep red blood cells at 70–80 ng/ml concentration range.

Chiropsalmus quadrigatus Toxin (CqTX-A)

A labile proteinaceous toxin CqTX-A (44 kDa) was isolated from another box jellyfish, *Chiropsalmus quadrigatus*, using a modified procedure developed for the CrTXs and CaTXs (Nagai et al. 2002). The deduced amino acid sequence of CqTX-A contained 462 amino acid residues and despite this showed no significant similarity to the CrTXs and CaTXs, with only 25.2 % and 21.6 % sequence homology, respectively.

The toxicity of CqTX-A showed an LD_{50} in crayfish of 80 μg/kg and an EC_{50} for hemolysis of sheep red blood cells of 160 ng/ml. These toxicities were less than for the CrTXs (5 μg/kg and 1.9 ng/ml, respectively) and CaTXs (5–25 μg/kg and 70 ng/ml, respectively). However, stinging by *C. quadrigatus* is more dangerous than stinging by *C. rastoni* or *C. alata* because the tentacles of *C. quadrigatus* are approximately three times more massive and longer than that of *C. rastoni* or *C. alata*. Since CqTX-A is the major toxin in the nematocysts of *C. quadrigatus*, it is likely the causative toxin in fatal cases of stings.

Chironex fleckeri Toxins (CfTXs)

The toxicity of *C. fleckeri* venom is known to be myotoxic, hemolytic, dermonecrotic, and lethal (Tibballs 2006). Two of the most abundant nematocyst proteins found in the box jellyfish, *Chironex fleckeri*, were identified as *C. fleckeri*

toxin-1 (CfTX-1) and toxin-2 (CfTX-2) (Brinkman et al. 2007). CfTX-1 and CfTX-2 sizes were estimated as 43 and 45 kDa through SDS-PAGE of purified nematocyst proteins. The zones for these proteins were transferred onto PVDF membranes for peptide sequencing and identified by N-terminal Edman degradation amino acid sequence determination. Internal amino acid sequences were identified by MALDI-TOF/TOF mass spectrometry (Australian Proteome Analysis Facility), and these sequences were used to confirm the amino acid sequence deduced from the cDNAs. CfTX-1 and CfTX-2 had 20 and 17-residue signal peptides and 436 and 445 amino acids for the complete open reading frame, respectively. Pairwise sequence alignment analysis of the CfTXs showed significant homology with CqTX-A (89 % and 72 %, respectively), CrTXs (27 % and 26 %, respectively), and CaTXs (24 % and 25 %, respectively).

Two more venomous proteins were purified from *C. fleckeri* nematocysts by size exclusion chromatography and cation exchange chromatography and named CfTX-A and CfTX-B, respectively (Brinkman et al. 2014). The sizes of these two toxins were slightly smaller (40 and 42 kDa, respectively) than that of CfTX-1 and CfTX-2. CfTX-A and CfTX-B toxins had 18 and 24-residue signal peptides and 436 and 437 amino acid residues for a complete open reading frame, respectively. Interestingly, CfTX-A and CfTX-B showed high similarity with CaTX-A (52–55 % identity) and not with CfTX-1 and CfTX-2 (23–25 % identity). The toxicities of CfTX-A and CfTX-B were related to an expanding family of lethal, pore-forming cnidarian toxins.

Structural Analysis of Jellyfish Toxins

Analysis of jellyfish toxins by MEMSAT3 and MEMSAT-SVM (http://bioinf.cs.ucl.ac.uk/software_downloads) classified the toxins into two groups by number of helix domains (Fig. 2a), named as class I and class II, respectively. As a result, the signal peptide (SignalP 4.1 server) and toxin hemolysis precursor (InterProScan server) were included in the coding sequence. Moreover, jellyfish toxins have pore-lining helix domains which are important to make a pore in the target cell membrane. In class I toxins, the pore-lining helix exists as one part, but in class II toxins, it is divided into two helices. These results support the hypothesis that jellyfish toxins are pore-forming toxins. However, it is not yet known how many jellyfish pore-forming molecules are needed to assemble for making the pore. Generally, pore-forming toxins have some amphipathic alpha-helix structure. By helix structure analysis, jellyfish toxins have critical amphipathic sequences in both.

Classes (Fig. 2b–d). While class I toxins are predicted to make alpha-helix structure with 30 amino acids, class II toxins make helix with 20 amino acids. There are 3.6 amino acid residues per turn. In general, it is suggested that jellyfish toxins secreted from nematocysts after reaching the target cells of the victim, such as red blood cell, make pores after self-assembly. The target cell will swell following the exchange of electrolytes through these pores, which results in osmotic lysis.

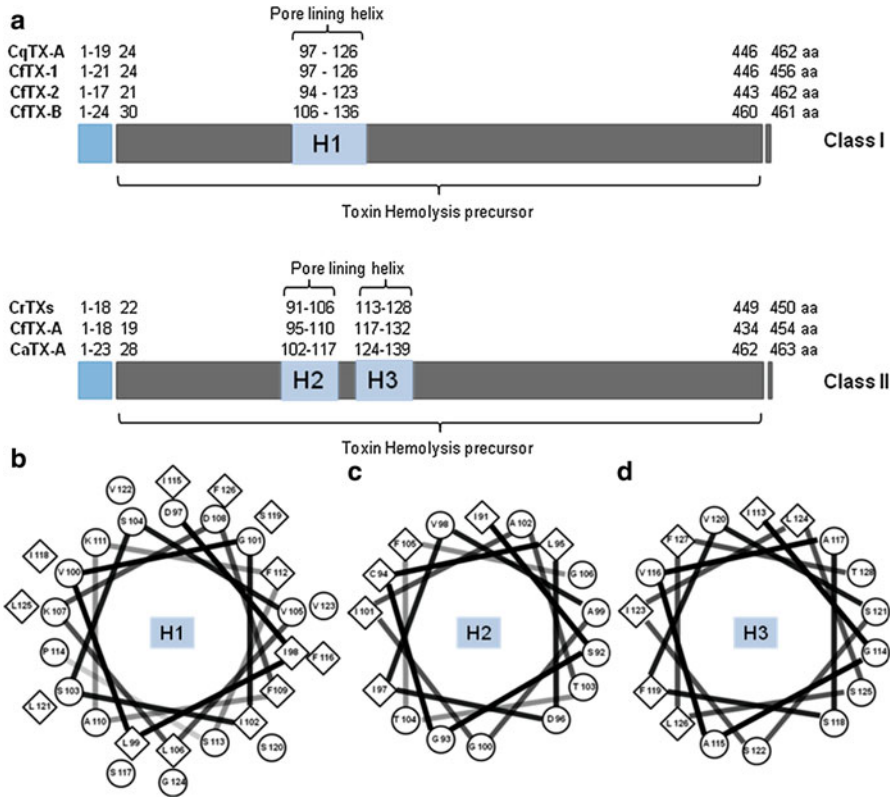

Fig. 2 Structural organization and amphiphilicity analysis of class I and class II jellyfish toxins. (**a**) Signal peptides (SignalP 4.1 server) are indicated in *blue*; putative pore-lining helices (MEMSAT3 and MEMSAT-SVM server) and toxin hemolysis precursor (InterProScan server) are indicated *up* and *below*, respectively. The sequence accession numbers are CrTXs (*Carybdea rastoni*: AB015878.1), CfTX-A (*Chironex Fleckeri*: JN695597.1), CfTX-B (*Chironex Fleckeri*: JN695598.1), CfTX-1 (*Chironex Fleckeri*: EF636902.1), CfTX-2 (*Chironex Fleckeri*: EF636903.1), CqTX-A (*Chiropsalmus quadrigatus*: AB045319.1), and CaTX-A (*C. alata*: AB036714.1). (**b–d**) represent putative helix sequences for CfTX-1 (from Asp-97 to Phe-126), CrTXs (from Ile-91 to Gly-106), and CrTXs (from Ile-113 to Thr-128), respectively. Hydrophobic residues are diamonds and hydrophilic residues are *circles*

Evolutionary Relationships of Jellyfish Toxins

Brinkman et al. (2014) classified box jellyfish toxins into two groups (type I and type II). CqTX-A, CfTX-1, and CfTX-2 were classified as type I and the other (CrTX-A, CaTX-A, CfTX-A, and CfTX-B) as type II by phylogenetic analysis. Both groups are predicted to contain signal peptides and form dual-domain mature proteins. Interestingly, only the type II toxins have short propart residues followed by a classical dibasic proteolytic cleavage site (RR/KR) between the signal peptide

and mature protein. In general, N-terminal proparts have important roles in protein biosynthesis, activation, correct folding, and posttranslational modification. Moreover, the proparts play a role in the activation of protoxins to toxins by the cleavage proparts. Regardless, all of the box jellyfish toxins have lethal and hemolysis toxicities, and type II toxins elicit more potent hemolytic activity than type I toxins. However, type I toxins elicit more potent cardiovascular effects in vertebrates than type II toxins (McClounan et al. 2012; Saggiomo et al. 2012).

Treatments of Stings

Jellyfish stings in humans are usually painful and the symptoms of envenomation can be classified as local and systemic effects (Cegolon et al. 2013). The former may involve local skin damage (necrosis) with erythema, blister formation, pruritus, and then scar formation. When a significant amount of venom reaches the circulation, the sting can be accompanied by systemic pain, hypertension, cardiotoxicity, pulmonary edema, anaphylaxis, and even death. In general, the stings are usually not considered life threatening, except those occurring by box jellyfish species (e.g., *Chironex fleckeri*) in tropical areas. In Australia, *C. fleckeri*-associated human deaths have been relatively well documented. Although there has been a significant amount of research effort to optimize the treatment of cnidarian stings, confusion still exists as to what is the most effective first aid and subsequent clinical management. Therefore, it is urgently desirable to develop a general protocol for the management of these accidents.

The treatment of jellyfish stings has traditionally focused on two strategic goals, the relief of pain by deactivation of nematocysts and the neutralization of venom (Ostermayer and Koyfman 2015). Once jellyfish venom is injected into the skin, the use of its antivenom (in case of severe envenomation) usually by medical personnel is the only treatment that can neutralize and deactivate the venom constituents that have entered the body. Therefore, in a majority of jellyfish sting cases, the treatment mainly depends on the relief of pain by deactivation of nematocysts, namely, topical management of affected skin areas. In fact, there are numerous folk remedies proposed as a first aid of jellyfish stings. However, many of them lack scientific evidence and in some stings may accidently result in a worsened condition. It is hardly a matter for surprise to find different international organizations recommending different first-line treatments. The International Life Saving Federation suggests topical ice packs for pain relief for all stings and topical acetic acid (4–5 %) for deactivation of remaining nematocysts only in box jellyfish stings (Fenner 2000). However, there is no single protocol or golden standard for the first aid of jellyfish stings. Nematocysts from different species of venomous jellyfish may respond to the same stimulant in an opposite manner. Therefore, it appears that treatments should be customized depending on the jellyfish species of envenomation (Honeycutt et al. 2014). The Australian Resuscitation Council (2010) recommendations for treating envenomation by jellyfish stings provide geographic guidelines based on tropical Australian (box and Irukandji-causing species) and

nontropical stings. For example, tropical box jellyfish (Cubozoa) stings can be treated with topical acetic acid (4–5 %) for deactivation of remaining nematocysts, whereas nontropical stings (e.g., Scyphozoa such as *Cyanea capillata*, Hydrozoa such as P*hysalia physalis*) should not be treated with acetic acid, which causes discharge of nematocysts. After immediately removing tentacles from the affected skin area, seawater is likely to be a good candidate for the first aid rinsing solution of all jellyfish stings regardless of their species differences, since, unlike other rinsing solutions, it does not stimulate discharges (Cegolon et al. 2013).

Potential Uses

Jellyfish venom is a research subject of great interest because many of these components may have pharmacological activities with therapeutic potential and some of them might be further developed as future drugs. However, in the last decade, despite the abundance and ubiquity of these animals, research on jellyfish venom has been limited by availability of samples and by difficulties in isolating pure venom. This may explain why the possible potential application of jellyfish venom for the therapy of human diseases has been poorly characterized in literature. With growing efforts and advances in high-throughput methods used for screening and characterizing bioactive compounds, jellyfish venom is now being studied for its promise as a therapeutic agent. Recently, research on jellyfish venom has focused on the identification of pharmacological activities and purifying active components as a new source of developing drugs. Antitumor activity has been the major area of interest in the screening of jellyfish venom.

Antitumor Activity

Reports on the cytotoxicity of jellyfish venom have been published since the early 1980s on cultured cells. Fractionated venom peptide (9 kDa) from *Chrysaora quinquecirrha* was observed to produce morphological changes in HEp2 and HeLa cells, to induce apoptosis with activation of caspase-8 and caspase-3, and to cause the downregulation of Bcl-2 and the fragmentation of DNA (Balamurugan et al. 2009). Isolated venom peptide of *Chrysaora quinquecirrha* was also shown to have an antitumor and antioxidant effects in the Ehrlich ascites carcinoma (EAC) tumor model. This venom peptide increased the survival time of the EAC-bearing mice by free radical scavenging property (Balamurugan et al. 2010).

Venom from the box jellyfish *Chiropsalmus quadrigatus* was shown to affect the growth of human U251 and rat C6 malignant glioma cells and transformed vascular endothelial ECV 304 cells. The isolated toxin (CqTX) from *C. quadrigatus* venom induced breaking of DNA bands, leading to apoptotic cell death. In particular, the expression of p53, a well-known tumor suppressor gene, was increased in U251 cells. It was suggested that treatment with CqTX could induce apoptosis in glioma

cells due to regulation of p53 expression, suggesting a possible application of apoptosis-inducing venom in glioma therapy (Sun et al. 2002).

Recently, *Pelagia noctiluca* venom demonstrated antitumoral activity in human colon cancer HCT 116 cells (EC_{50} 320 μg/ml), neuroblastoma SH-SY5Y cells, and glioma U87 cells (EC_{50} 180 μg/ml). Its venom exhibited potent cytotoxicity, increasing ROS and catalase levels and inducing lipid peroxidation in HCT 116 cells. These results indicate that this venom causes cytotoxicity through oxidative stress (Ayed et al. 2011). Another group studied the effect of *P. noctiluca* crude venom in human neuroblastoma SH-SY5Y cells. Similar to the previous study, its crude venom was shown to induce oxidative stress with disruption of the mitochondrial membrane, inhibition of mitochondrial respiration, and uncoupling of oxidative phosphorylation (Morabito et al. 2012). Furthermore, crude or fractionated *P. noctiluca* venom reduced the viability and adhesion to fibrinogen in U87 cells. Among four fractions, F1, F2, and F3 showed significant antiproliferative activity in a time-dependent manner. Although the interaction of the venom with specific integrins was not investigated in the study, the authors stated that integrins αVβ3 and α5β1 are involved in the antitumor activity induced by *P. noctiluca* venom. Purification and determination of chemical structures of these active fractions are under investigation (Ayed et al. 2012a).

Cotylorhiza tuberculata, "the fried egg jellyfish," is known to be a non-dangerous jellyfish throughout the world. However, its medusa extract was studied for cytotoxicity against human breast cancer MCF-7 cells and epidermal keratinocyte HEKa cells at concentrations raging from 0.0005 to 0.08 μg/ml. *Cotylorhiza* extracts were found to be remarkably cytotoxic and reduced the viability of MCF-7 cells but not nonmalignant HEKa cells. A remarkable time- and dose-dependent enhancement of gap junction intracellular communication (GJIC) was evident in MCF-7 cells treated with jellyfish extract (Leone et al. 2013).

Insecticidal Activity

Proteinaceous venom from tentacles of *Rhopilema esculentum* Kishinouye showed different insecticidal activity against three common pest species in China, *Stephanotis pyri* Fabricius, *Aphis medicaginis* Koch, and *Myzus persicae* Sulzer. Of the three pests, *S. pyri* was the most sensitive to *R. esculentum* venom; thus, the author stated that *S. pyri* could be a potential "target pest" for this venom. They investigated the purification of insecticidal proteins and their mechanisms of action (Yu et al. 2005). The insecticidal activity of jellyfish toxins was not studied before this study.

Insecticidal activity of crude (SFV and NSFV) and fractionated venom (Fr-1, Fr-2, NFr-1, and NFr-2) from *Stomolophus meleagris* were investigated for antiproliferative effect against the cotton bollworm *Helicoverpa armigera*, one of the most widely distributed Noctuidae worldwide. SFV, NFr-1, and NFr-2 showed inhibitory effects on the growth of *H. armigera,* while SFV, Fr-1, Fr-2, NRFV, and

NFr-1 had no or low insecticidal activity against the neonate larvae of *H. armigera*. Of the six samples, NFr-2 had the strongest insecticidal activity against the neonate larvae of *H. armigera*, and the estimated mortality recorded at 7 days of NFr-2 was 74.23 % (Yu et al. 2014).

Antihypertensive Activity

Jellyfish venom has long been recognized for its cardiovascular effects. Most cubozoan jellyfish species induce hypertension, while scyphozoan jellyfish induce hypotension and bradycardia (Brinkman and Burnell 2009; Kim et al. 2006). It is conceivable that hypotensive activity induced by jellyfish venom can be used as a biological source of antihypertensive agents. To date, it was reported that two identified peptides (VKP and VKCFR) derived from *R. esculentum* hydrolysate showed angiotensin-converting-enzyme (ACE) inhibitory activity and protection effect in a H_20_2-induced endothelial cell injury model by enhancing antioxidant enzyme activity and quenching the hydroxyl radical (Li et al. 2014).

Anticoagulant Activity

Aurelia aurita tentacle extract was studied for its anticoagulant activity in vitro. The jellyfish tentacle extract (JFTE) showed very strong fibrinogenolytic activity, cleaving the α and β chains from the fibrinogen molecule. The fibrinogenolytic activity was found to be stronger than for some snake venom-derived anticoagulants. JFTE also completely liquefied fibrin clots in 24 h. The fibrinogenolysis appears to be caused by high molecular weight fractions of the extract. PMSF, a serine protease inhibitor, significantly reduced fibrinogenolytic activity, and heating totally abolished it (Rastogi et al. 2012).

Analgesic Activity

Pelagia noctiluca crude venom and its two fractions exhibited analgesic and anticholinesterase activities, inhibiting nonspecific cholinesterase (BuChE) at different doses without acute toxicity. The three venom samples showed significant analgesic effects compared to the analgesic drug, acetylsalicylate of lysine (ASL). Fraction 2 was found to exhibit higher analgesic activity than fraction 1. Furthermore, cholinesterase (ChE) hydrolyzes acetylcholine (ACh), the neurotransmitter at cholinergic synapses. Inhibition of ChE is one of the strategies for the treatment of Alzheimer's disease, senile dementia, ataxia, myasthenia gravis, and Parkinson's disease (Atta-ur-Rahman and Choudhary 2001; Karlsson et al. 1984). Similar to analgesic activity, fraction 2 also exhibited more anti-BuChE activity (Ayed et al. 2012b).

Conclusion and Future Directions

Jellyfish are some of the most hazardous sea creatures. Due to their toxicity, in general, the jellyfish toxins induce intense pain and swelling with surrounding redness of the skin. However, despite the research efforts of the past several decades, little is known about the toxicological effects of jellyfish toxins, which remain a mystery, including their mechanisms of action at the molecular level. Also, the biological roles of these jellyfish toxins need to be investigated to provide a more comprehensive understanding of their physiological and pathological properties.

More recently, various animals which have toxins, such as snakes, scorpions, cone snails, and spiders, have become attractive to pharmaceutical researchers in search of new drugs. Certainly, jellyfish venoms are attractive candidates for clinical applications (Turner et al. 1969). The exact mechanism of action of toxins is still unclear. However, several jellyfish species may serve as leads for new drug development in cases such as arthritis, cardiovascular medicine, and other disorders. This facet may be of interest to scientists working on these toxins.

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Cross-References

- ▶ Clinical and Therapeutic Aspects of Envenomations Caused by Sponges and Jellyfish
- ▶ Miscellaneous Marine Toxins of Medical Significance
- ▶ Phylum Porifera and Cnidaria
- ▶ Toxins Produced by Marine Invertebrate and Vertebrate Animals: A Short Review

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